

IN THE CLAIMS

Please amend the claims as follows:

Claim 1 (Currently Amended): A method for detecting, separating and identifying an expressed trace protein and/or peptide in a test sample, comprising:

converting wherein a protein and/or peptide in a test sample is converted to a fluorescent derivative,

separating said fluorescent derivative is separated by a combination of HPLC and fluorescence detection (HPLC/fluorescence detection) to obtain fluorescent fractions,

applying the fluorescent fractions fraction is applied to mass spectrometry or MS/MS analysis, or applying the fluorescent fractions fraction is applied to enzymatic hydrolysis to obtain peptide fragments,

separating the peptide fragments are separated to obtain peptide fractions, and applying the peptide fractions are applied to mass spectrometry or MS/MS analysis, collating the mass spectrometry or MS/MS data collated with a database, and providing said collated data provided for structural analysis to identify the expressed protein and/or peptide.

Claim 2 (Currently Amended): The method according to claim 1, wherein after converting the protein and/or peptide in the test sample to a fluorescent derivative, the fluorescent derivative is applied to HPLC to capture a the fluorescent fraction, the fluorescent fraction is applied to enzymatic hydrolysis, and fluorescence-labeled fragments and non-fluorescence-labeled fragments are applied to mass spectrometry or MS/MS analysis, and the ion molecular weight data of each of the fragments thus obtained is collated with a protein and/or peptide fragment database for structural analysis.

Claim 3 (Currently Amended): The method according to claim 1, wherein (1) the protein and/or peptide in the test sample is labeled with a fluorescence reagent, (2) the fluorescent fraction is captured by subjecting the labeled protein and/or peptide to one-dimensional or two-dimensional HPLC/fluorescence detection, (3) the fluorescent fraction is applied to enzymatic hydrolysis, and (4) together with obtaining a fluorescence chromatogram is obtained by second stage HPLC/fluorescence detection of the resulting

hydrolysis product, and all of the peaks are applied to mass spectrometry ~~and collated with a database for structural analysis.~~

Claim 4 (Previously Presented): The method according to claim 1, wherein a functional group-specific fluorescence reagent is added to an aqueous solution of the protein and/or peptide sample, and a surfactant and/or protein denaturing agent is optionally added, to fluorescently label the protein and/or peptide.

Claim 5 (Currently Amended): The method according to claim 1, wherein the ~~fluorescence-labeled protein and/or peptide sample~~ fluorescent derivative is applied to a separation means selected from the group consisting of an typified by ion exchange column HPLC equipped with a fluorescence detector, a reverse phase partition HPLC, a gel filtration HPLC, and [[or]] electrophoresis, and a [[the]] peak fraction thereof is captured while monitoring fluorescence.

Claim 6 (Currently Amended): The method according to claim 1, wherein the fluorescent fraction is subjected to enzymatic hydrolysis using a protease selected from the group consisting of a peptidase, a trypsin and a chymotrypsin typified by various types of peptidases, trypsins and chymotrypsins.

Claim 7 (Previously Presented): The method according to claim 1, wherein the enzymatic hydrolysis product is applied to reverse phase HPLC equipped with a fluorescence detector to detect a fluorescence peak, and mass spectrometry or MS/MS analysis is carried out on fluorescence-labeled fragments and non-fluorescence-labeled fragments.

Claim 8 (Canceled).

Claim 9 (Previously Presented): The method according to claim 1, wherein the test sample is a protein and/or peptide sample collected from a biological sample.

Claim 10 (Previously Presented): The method according to claim 1, wherein database collation is carried out using a database containing protein and/or peptide fragment data and fluorescent reagent-labeled amino acid data.

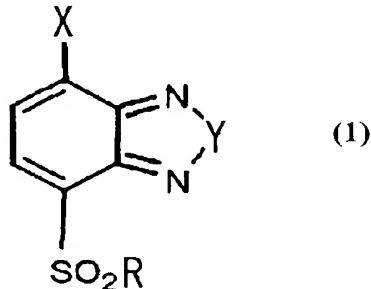
Claim 11 (Currently Amended): A system for detecting, separating and identifying an expressed trace protein and/or peptide used in the method according to claim 1 comprising, ~~as constituent elements thereof~~, a first reactor for labeling a protein and/or peptide of a test sample with a fluorescence reagent, a one-dimensional or two-dimensional HPLC equipped with a fluorescence detector for fluorescent fractionation of a fluorescent derivative labeled with the fluorescence reagent, a second reactor for enzymatic hydrolysis of the fluorescent fraction, a second-stage HPLC equipped with a fluorescence detector for fluorescent detection of fluorescence-labeled fragments of the enzymatic hydrolysis product, and one or two or more types of structural analysis devices equipped with a database containing data on amino acids labeled with the fluorescence reagent.

Claim 12 (Original): The system according to claim 11, wherein the first reactor, the one-dimensional or two-dimensional HPLC equipped with a fluorescence detector, the second reactor, and the second-stage HPLC equipped with a fluorescence detector are arranged in series.

Claim 13 (Currently Amended): The method according to claim 1, wherein a protein and/or peptide in a test sample is converted to a fluorescent derivative by using as a fluorescent derivatization reagent

a compound represented by the following general formula (1):

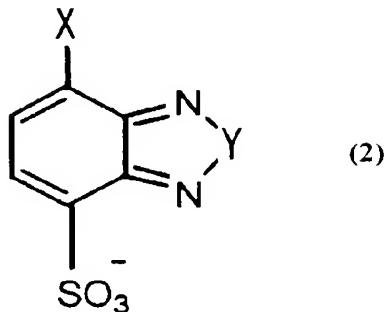
[Formula 1]



wherein (wherein, X represents a halogen atom, Y represents O, Se or S, and R represents -NH₂, -NHR' (wherein, R' represents an alkyl-substituted N-alkyl group, dialkyl-substituted N-alkyl group or trialkyl-substituted N-alkyl group) or -NR"R'" (wherein, R" represents an alkyl group, and R''' represents an alkyl-substituted N-alkyl group, dialkyl-substituted N-alkyl group or trialkyl-substituted N-alkyl group)[()]) or an isotope compound thereof, or

a compound represented by ~~the following general formula (2):~~

{Formula 2}

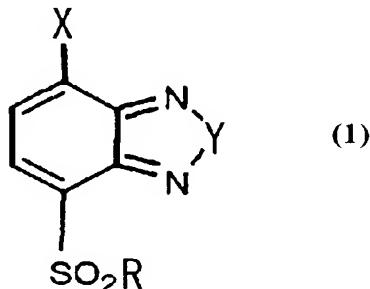


wherein (wherein, X represents a halogen atom and Y represents Se or S), S, or an isotope compound thereof.

Claim 14 (Currently Amended): The method according to claim 1, wherein a protein and/or peptide in the method according to claim 1 is converted to a fluorescent derivative by using a fluorescent derivatization reagent used for fluorescent derivatization which is

a compound represented by ~~the following general formula (1):~~

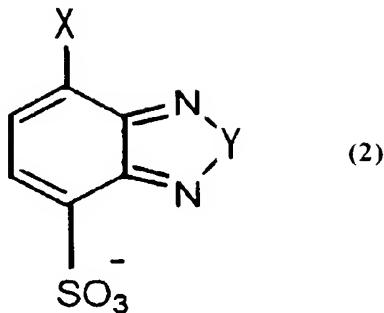
{Formula 3}



wherein (wherein, X represents a halogen atom, Y represents O, Se or S, and R represents - NH_2 , - NHR' (wherein, R' represents an alkyl-substituted N-alkyl group, dialkyl-substituted N-alkyl group or trialkyl-substituted N-alkyl group) or - $\text{NR}''\text{R}'''$ (wherein, R'' represents an alkyl group, and R''' represents an alkyl-substituted N-alkyl group, dialkyl-substituted N-alkyl group or trialkyl-substituted N-alkyl group)[()]) or an isotope compound thereof, or

a compound represented by ~~the following general formula (2):~~

{Formula 4}



wherein (wherein, X represents a halogen atom and Y represents Se or S, S), or an isotope compound thereof.

Claim 15 (Original): A method for detecting, separating and identifying a protein and/or peptide, wherein a protein and/or peptide of a test sample is converted to a fluorescent derivative, the fluorescent derivative is separated and detected with an HPLC, enzymatic hydroxylation is carried out following fractionation, and sequence analysis and protein identification are carried out by direct mass spectrometry of the hydrolysis product.

Claim 16 (Original): A method for detecting, separating and identifying a protein and/or peptide, wherein a protein and/or peptide in different test samples in the form of sample A and sample B is converted to a fluorescent derivative, respectively, with at least two fluorescent derivatization reagents having different fluorescence wavelengths, the fluorescent derivative is separated and detected with an HPLC equipped with a fluorescence detector, and identification is carried out by applying to quantification of each fluorescence peak either directly or collectively following fractionation and/or applying each fluorescence peak collectively to enzymatic hydrolysis followed by quantification of the hydrolysis product, or applying the hydrolysis product to HPLC-mass spectrometry.

Claim 17 (Original): The method according to claim 16, wherein each fluorescence peak is applied to quantification by HPLC either directly or collectively, and the ratio of each derivative of the protein and/or peptide in sample A and sample B is calculated.

Claim 18 (Original): The method according to claim 16, wherein the hydrolysis product is applied to quantification by HPLC, and the ratio of each derivative of the protein and/or peptide in sample A and sample B is calculated.

Claim 19 (Original): The method according to claim 16, wherein the reaction product of a first fluorescent derivatization reagent and the reaction product of a second fluorescent derivatization reagent with the protein and/or peptide in sample A and sample B are combined, applied to two HPLC capable of excitation and fluorescence detection, applied to enzymatic hydrolysis after fractionating and combining each fluorescence peak, and identification is carried out by applying the hydrolysis product to HPLC-mass spectrometry.

Claim 20 (Original): The method according to claim 16, wherein samples A and B are two types of cell, tissue or body fluid samples.

Claim 21 (Currently Amended): The method according to claim 16, wherein the protein and/or peptide is converted to a derivative with at least two fluorescent derivatization reagents having different excitation and fluorescence wavelengths selected from the group consisting of among DAABD-X, DAASeBD-X and DAAThBD-X (wherein X represents Cl or F) DAABSeD-X, DAABThD-X, an isotope of DAABD-X, an isotope of DAABSeD-X, and an isotope of DAABThD-X, wherein X represents Cl or F.

Claims 22-23 (Canceled).

Claim 24 (Original): An automated fractionation device capable of fractionating a protein and/or peptide derivatized with a fluorescent derivatization reagent without degrading the protein and/or peptide, at least provided with a microcolumn HPLC, microfluorescence detector, microfraction collector and automated microinjector.

Claim 25 (Original): A high-performance, easily quantifying trace protein identification and analysis device, at least provided with a microcolumn HPLC, microfluorescence detector, microfraction collector, enzyme reaction device and automated microinjector, and optionally provided with a mass spectrometry (MS) system.

Claim 26 (New): The method according to claim 1, wherein after separating the fluorescent derivative by HPLC/fluorescence detection to obtain fluorescent fractions, the fluorescent fractions are applied to mass spectrometry or MS/MS analysis.